SUPPLEMENTARY MATERIAL

Macrophage metabolic adaptation to heme detoxification involves CO-dependent activation of the pentose phosphate pathway

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Supplementary Methods

Chemical Reagents

Hemin BioXtra (51280), cobalt protoporphyrin IX chloride (C1900), Oligomycin A (75351), Antimycin A (A8674), rotenone (R8875), 2-deoxy-D-glucose (D8375), and tricarbonylchloro(glycinato)ruthenium (II) (CORM-3) (SML0496) are from Sigma Aldrich (St. Loius, MO). Dehydroepiandosterone (2172-100) is from BioVision (Milpitas, CA). BAM15 (17811), tin protoporphyrin IX chloride (16375), and 6-aminonicotinamide are from Cayman Chemical (Ann Arbor, MI). Lipopolysaccharide (TLRL-3PELPS) is from InvivoGen (San Diego, CA).

Antibodies

For Western blots, mouse anti-PGD (abcam 129199), anti-G6PD (Bethyl Laboratories A300-404A) and anti-β-actin (LI-COR Biosciences 926-42210) were used as primary antibodies. For FACS, fluorophore-conjugated antibodies anti-CD45-APCeFluor780 (eBioScience), anti-CD11b-FITC (BD Biosciences), and anti-F4/80-PECy7 (eBioScience) were used.

Heme and CORM3 Solution Preparation

Heme solutions for *in vitro* experiments were prepared immediately prior to administration by dissolving hemin in NaOH (20mM in water) to a stock concentration of 2mM. The stock solution was protected from light and sterile filtered prior to cell treatment, and discarded after use. Inactivated CORM3 (iCORM3) was prepared by dissolving the appropriate amount of CORM3 into the experimental medium and allowing for 24 hours for the carbon monoxide to dissipate.

Metabolomics

Unbiased metabolomics analyses were performed using a liquid chromatography-mass spectrometry based platform developed by Metabolon, Inc., (Durham, NC). Previous details of sample preparation, UPLCMS conditions, quality control and data analysis are described previously[1] and briefly summarized here. Protein was precipitated from samples by vigorous shaking in methanol and subsequent centrifugation. The remaining extract was divided into fractions for independent analyses (2 reverse-phase positive ion mode electrospray UPLC-MS/MS, 1 reverse-phase negative ion mode electrospray UPLC-MS/MS, and 1 HILIC/UPLC-MS/MS in negative ion mode on Thermo Scientific Q Exactive high-resolution mass spectrometer with heated electrospray ionization (HESI-II) source and Orbitrap mass analyzer coupled to a Waters ACQUITY ultra-performance liquid chromatograph). In all cases multiple internal reference standards were included to correct for instrument variability and perform chromatographic alignment. Data analysis was

performed as previously described[1]. Metabolite levels were normalized to sample protein content (measured by Bradford Assay), and differentially abundant analytes between control and heme-loaded macrophages was determined by Welch's unpaired t-test. A complete list of significantly altered metabolites is presented in Supplementary Table 2

Extracellular Flux Analysis

Mitochondrial Stress Test: At the beginning of the assay, the media was changed to DMEM with pyruvate (Thermo-Fisher 12800017, pH=7.35 at 37°C) and cells equilibrated for 30 minutes. Oxygen consumption rate (OCR) from the cell media was measured using four-minute measurement periods, represented in units of pmol O2/min, using a Seahorse XF24 Flux Analyzer (Agilent Technologies, Santa Clara, CA). After three basal OCR measurements, compounds to modulate cellular respiratory function: 1μM Oligomycin (to inhibit ATP Synthase); 2μM BAM15 (to cause mitochondrial uncoupling); 1μM Antimycin A & 100nM Rotenone (to inhibit mitochondrial respiration)] were injected into the plate, after every three measurements, in order. Basal respiration was calculated by subtracting the average of the post-Antimycin A and Rotenone measurements from the average of the first three measurements. Maximum respiratory capacity was calculated by subtracting the average of the post- Antimycin A and Rotenone measurements from the average of the post-BAM15 measurements. The reserve capacity was calculated by subtracting the average of the basal measurements from the average of the post-BAM15 measurements.

Glycolytic Stress Test: At the beginning of the assay, the media was changed to GST media (unbuffered, glucose-free DMEM; Sigma-Aldrich D5030, pH=7.35 at 37°C; supplemented with 143mM NaCl and 2mM glutamine) has fewer buffering agents than the media used for the MST, allowing for greater sensitivity in measuring ECAR. After three basal ECAR measurements, compounds to modulate glycolysis [20mM glucose; 1μM Oligomycin (to inhibit mitochondrial ATP production); 80mM 2-deoxyglucose (to inhibit hexokinase)] were injected after every four measurements and ECAR was measured using three-minute measurement periods. Basal glycolysis was calculated by subtracting the average of the post-2-deoxyglucose measurements from the average of the post-Glucose measurements. Glycolytic capacity was calculated by subtracting the average of the post- 2-Deoxyglucose measurements from the average of the post-Oligomycin measurements.

[^{13}C]$_6$-Glucose Metabolite Tracing

RAW macrophages ($5x10^6$ cells) were plated on 6cm tissue culture treated dishes in complete medium (DMEM with 10% FBS, 2% HEPES, 2% Antibiotic-Antimycotic) and allowed to adhere overnight. Labeled glucose media was prepared by adding [^{13}C]$_6$-glucose (Sigma Aldrich) to glucose-free DMEM (Gibco) to a final concentration of 25mM. On

the day of the experiment, media was removed and cells were washed 3 times with sterile PBS, before vehicle or heme (10μM) in $[^{13}C]_6$-glucose media was added. After 6 hours, media was removed, and cells were placed at -80C for 15 minutes to slow cellular metabolism. Cells were then scraped into prechilled lysis buffer (80:20 HPLC-grade methanol:water) on dry ice, centrifuged at 20,000 x *g* for 30 minutes to remove membrane components, and the supernatant retained for LCMS analysis.

Sample extracts were dried down under vacuum before resuspending in water/acetonitrile (15:85) and subjected to chromatography. Chromatography was performed on Waters I-class Acquity unit with a 1.0 x 150mm Waters amide column held at 45°C. Mobile phase A consisted of water and acetonitrile (95:5 v/v) with 20mM ammonium acetate (pH 9) and mobile phase B was acetonitrile. At a flow rate of 0.12 ml/min, the gradient began at 85% B and proceeded to 32% B over 3.5 minutes before washing the column with 98% A and equilibration to starting conditions at 85% B. The eluate was analyzed with an in-line Waters TQ-S mass spectrometer operating in negative ion mode using electrospray ionization to monitor metabolites, including 13C isotopes, using multiple reaction monitoring.

Flow-Assisted Cell Sorting

Macrophage-like cells were isolated from spleens of control or SCD mice. Mice were euthanized my carbon dioxide inhalation and cardiac puncture, and the spleen was excised surgically, homogenized between the frosted surfaces of two glass slides, passed through a 70um filter and washed with sterile PBS. Cells were resuspended in 10ml of 0.83% ammonium chloride for 5 minutes to lyse erythrocytes, and remaining cells washed with PBS and resuspended in FACS buffer (PBS, 0.1% BSA, 2mM EDTA, 0.05% sodium azide) for fluorescent antibody staining. Cells were stained with Live/Dead Aqua (Invitrogen) according to the manufacturer's recommendations for 30 minutes, on ice and protected from light. Cells were washed three times with FACS buffer, then resuspended in FACS buffer containing anti-mouse CD16/CD32 (Biolegend) to block Fc receptors for 30 minutes. Cells were washed three times in FACS buffer and resuspended in FACS buffer containing surface stain antibodies, anti-CD45-APCeFluor780 (eBioScience), anti-CD11b-FITC (BD Biosciences), and anti-F4/80-PECy7 (eBioScience), and stained on ice, protected from light for 1 hour. Cells were washed three times with FACS buffer, and resuspended in FACS buffer for sorting on a FACSAria III (BD Biosciences) using unstained and single-stain controls. Cells were sorted into microcentrifuge tubes containing FACS buffer and placed on ice. Cells were pelleted by centrifugation, supernatant removed, and cells lysed in RLT buffer for RNA isolation.

CORM3 Administration and Peritoneal Cell Isolation

10 week-old male C57BL/6J (wild-type) mice were treated with either saline vehicle or CORM3 for 7 days. CORM3 was freshly dissolved in sterile 0.9% NaCl solution and administered by intraperitoneal injection at a dose of 10mg/kg body mass once daily for 7 days. At the end of the experiment, mice were euthanized by carbon dioxide inhalation and cardiac puncture, and abdominal skin dissected to expose the peritoneal cavity. Approximately 10ml of ice-cold, sterile PBS was injected into the peritoneal cavity, and lavage containing peritoneal cells collected. Cells were pelleted via centrifugation at 1500 x *g* for 5 minutes, the supernatant was removed, and cells were lysed into RLT buffer for RNA extraction.

Functional Cellular Assays

G6PD Activity: BMDMs were seeded at $2x10^6$ cells/well in 6-well plates and allowed to adhere overnight. After vehicle, heme, CORM, or iCORM treatment, cells were lysed on ice in RIPA buffer containing complete mini protease inhibitor, sonicated for 30 seconds, and centrifuged at 18000 x *g* to pellet cellular debris. The supernatant was transferred to a clean tube, and protein content determined by BCA Assay (Thermo Scientific). G6PD activity of the supernatant was measured using the Glucose-6-phosphate Dehydrogenase Activity Assay Kit (Cayman Chemical 700300) according to the manufacturer's instructions.

Glucose Uptake: BMDMs were seeded at $4x10^5$ cells/well in 96-well plates and allowed to adhere overnight. Cells were treated with vehicle (RPMI media containing no glucose), heme (50μM), or lipopolysaccharide (1μg/mL) for 6 hours. Glucose uptake was measured using the Glucose Uptake Cell-based Assay Kit (Cayman Chemical 600470), which measures the uptake of the fluorescent glucose analog 2-(N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)amino)-2-deoxyglucose, according to the manufacturer's instructions.

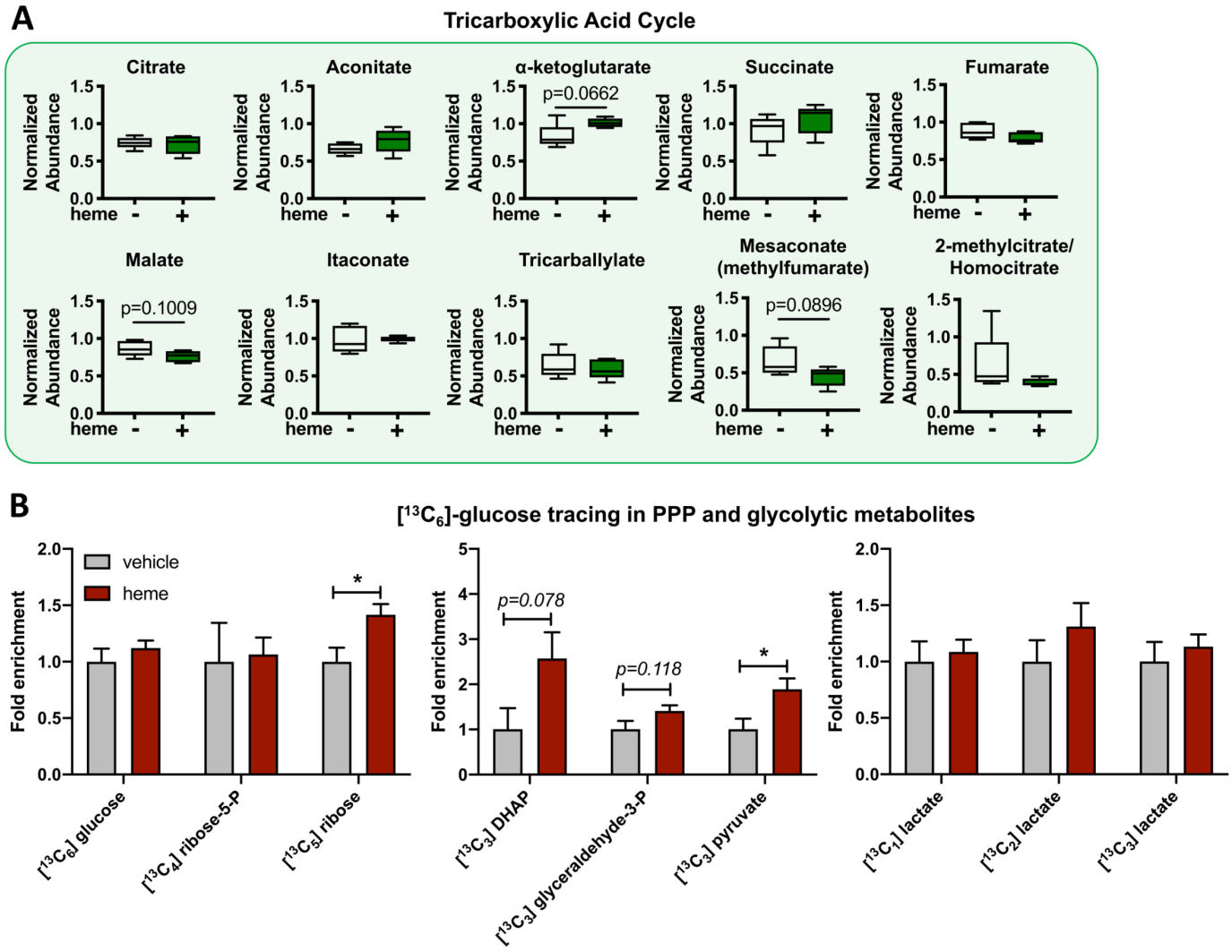
Intracellular Heme Content: BMDMs were seeded at $2x10^6$ cells/well in 6-well plates and allowed to adhere overnight. Cells were treated with vehicle, 10, 20, or 50 μM heme, and/or DHEA as appropriate. For degradation experiments, after the heme loading treatment, media was removed, cells washed twice with sterile PBS, and a heme-free medium containing vehicle or DHEA was added. At the time points indicated, media was removed under vacuum, cells were washed twice in cold PBS, and cells lysed with RIPA buffer. Samples were centrifuged at 18000 x g to pellet cellular debris, and the supernatant transferred to a clean tube. The heme concentration in the cellular supernatant was quantified using the QuantiChrom Heme Assay Kit (BioAssay Systems DIHM-250), and normalized to the supernatant protein level as measured by BCA assay (Thermo Scientific).

NADPH and GSH Content: BMDMs were seeded at $4x10^5$ cells/well in 24-well plates and allowed to adhere overnight. Cells were treated with vehicle, heme, iCORM3, or CORM3 for 6 hours. Intracellular NADPH measurement was done using the NADP/NADPH Quantitation Colorimetric Kit (BioVision K347). Levels of reduced and oxidized glutathione were determined using the GSH/GSSG-Glo Assay Kit (Promega V6611).

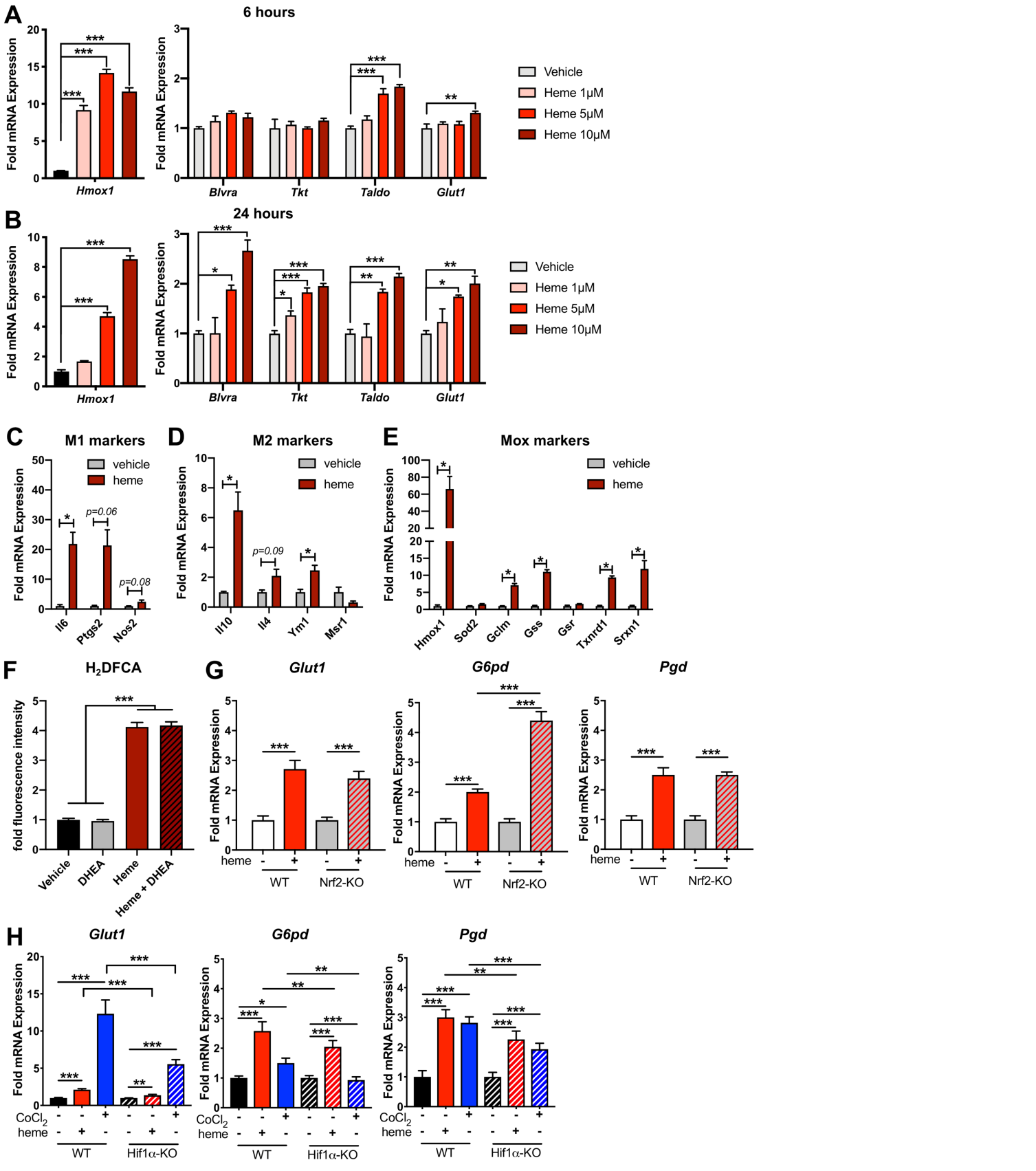
Intracellular ROS Assay: BMDMs were seeded at $1x10^5$ cells/well in clear-bottomed, black-walled 96-well plates and allowed to adhere overnight. Cells were treated with vehicle, heme, and/or DHEA for 6 hours, and ROS levels determined using the Reactive Oxygen Species (ROS) Detection Assay Kit (BioVision K936) following the manufacturer's instructions.

Intracellular ATP Content: BMDMs were seeded at $4x10^5$ cells/well in 24-well plates and allowed to adhere overnight. The media was replaced with one lacking serum, and allowed to recover for 1 hour, before vehicle, heme, or CORM3 was added. Immediately prior to treatment and at 2, 4, 6, and 8 hours, the media was removed by careful aspiration. Intracellular ATP was measured using the Cell Titer Glo Kit (Promega G7571) according to the manufacturer's instructions.

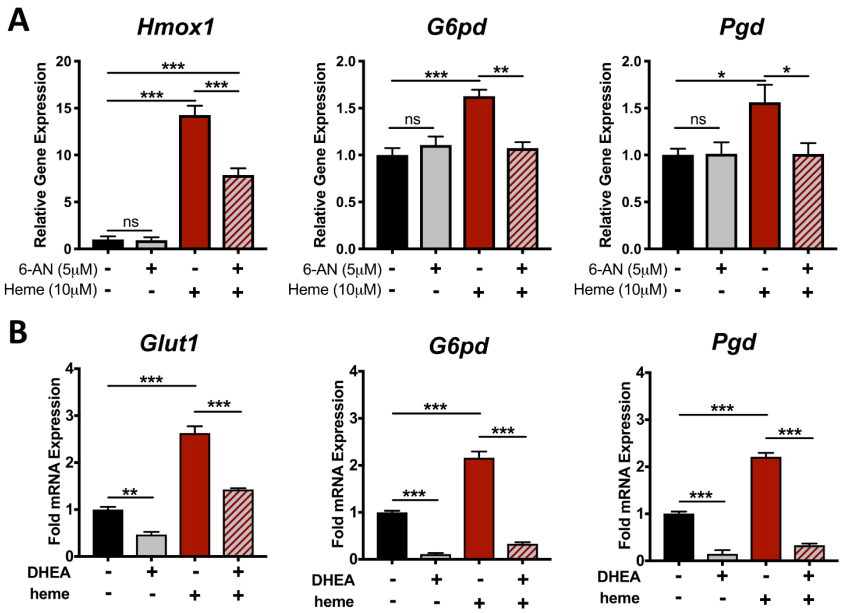
Supplementary Figures and Tables



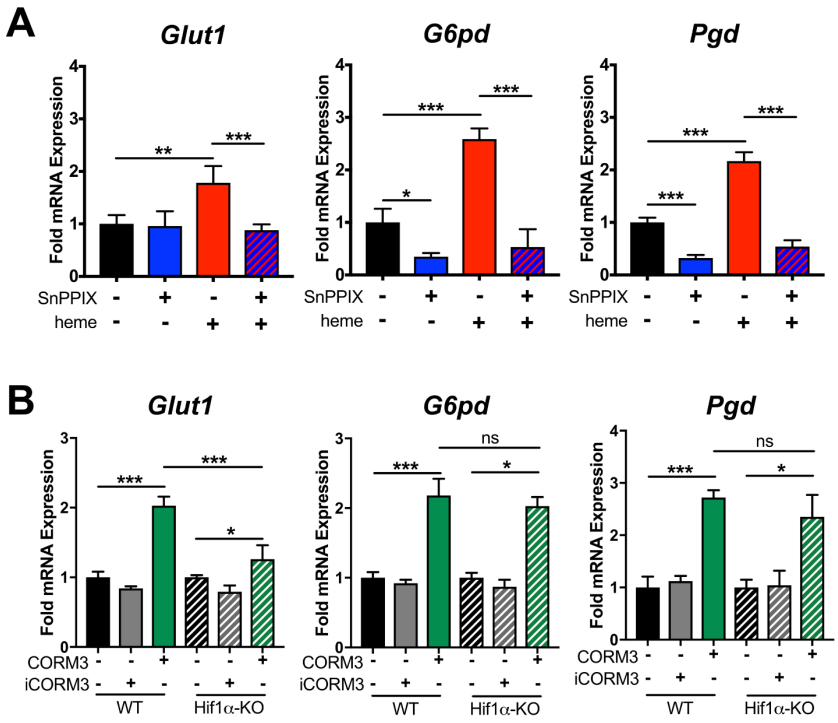
Supplementary Figure 1. Related to Figure 2. A. Box plots representing individual metabolites involved in the tricarboxylic acid (TCA) cycle measured in BMDMs treated with either vehicle or 10 μM heme for 6 hours (n = 5), showing no significant differences. Data are represented as boxplots displaying the median and range of relative abundance measurements for control and heme-loaded BMDMs (n = 5). Statistical significance between 2 groups was determined by Welch's unpaired t-test. **B.** $[^{13}C]_6$-glucose flux tracing studies of vehicle or heme-loaded RAW cells after 6 hours (n = 4). Data are mean ± SEM. * represents $p<0.05$ by Welch's unpaired t-test.


A
6 hours
Fold mRNA Expression
Hmox1
Blvra
Tkt
Taldo
Glut1
Vehicle
Heme 1μM
Heme 5μM
Heme 10μM
B
24 hours
C
M1 markers
vehicle
heme
p=0.06
p=0.08
Il6
Ptgs2
Nos2
D
M2 markers
p=0.09
Il10
Il4
Ym1
Msr1
E
Mox markers
Hmox1
Sod2
Gclm
Gss
Gsr
Txnrd1
Srxn1
F
H2DFCA
fold fluorescence intensity
Vehicle
DHEA
Heme
Heme + DHEA
G
Glut1
G6pd
Pgd
heme
WT
Nrf2-KO
H
Glut1
G6pd
Pgd
CoCl2
heme
WT
Hif1α-KO

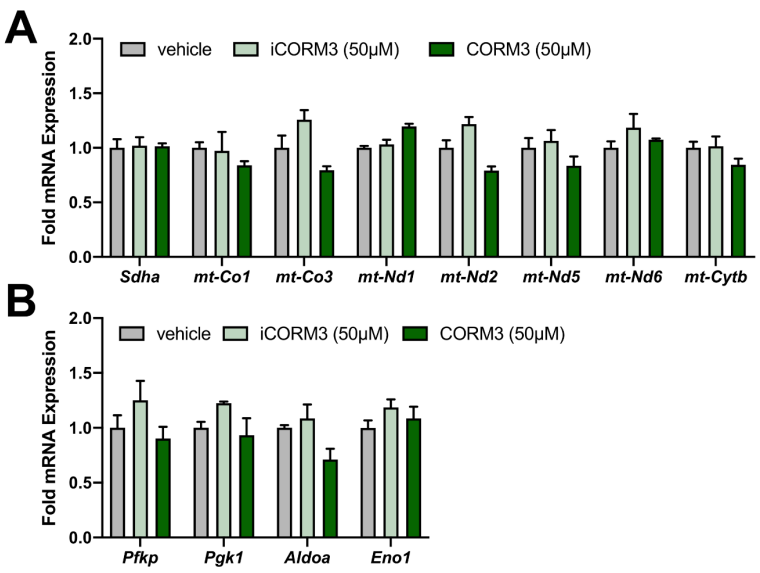
Supplementary Figure 2. Related to Figure 3. **A**. mRNA expression of heme clearance enzymes *Hmox1* and *Blvra*, *Glut1*, and pentose phosphate pathway enzymes *Tkt* and *Taldo* in BMDMs treated with 1-10μM heme for 6 hours (n = 4). **B**. mRNA expression of heme clearance enzymes *Hmox1* and *Blvra*, *Glut1*, and pentose phosphate pathway enzymes *Tkt* and *Taldo* in BMDMs treated with 1-10μM heme for 24 hours (n = 4). **C.** mRNA expression of 'M1' macrophage markers in BMDMs treated with vehicle or heme for 6 hours (n = 4). **D.** mRNA expression of 'M2' macrophage markers in BMDMs treated with vehicle or heme for 6 hours (n = 4). **E.** mRNA expression of 'Mox' macrophage markers in BMDMs treated with vehicle or heme for 6 hours (n = 4). **F**. Reactive Oxygen Species (ROS) levels, measured by H_2DFCA fluorescence, in macrophages treated with vehicle, heme, or DHEA for 6 hours (n = 6). **G.** mRNA expression of *Glut1*, *G6pd*, and *Pgd* in BMDMs isolated from wild-type (WT) or Nrf2-deficient mice loaded with vehicle or 10μM heme for 6 hours (n = 4). **H**. mRNA expression of *Glut1*, *G6pd*, and *Pgd* in BMDMs isolated from WT or Hif1α-deficient mice treated with vehicle, 10μM heme, or 100μM $CoCl_2$ (a Hif1α activator) for 6 hours (n = 4). Data are represented as mean ± SEM. Statistical significance between 2 groups was determined by Welch's unpaired t-test. Significance between more than 2 groups was determined by One-Way ANOVA, with post-hoc Tukey's or Dunnett's Multiple Comparison Tests, as appropriate, to determine differences between specific groups. * represents $p<0.05$, ** represents $p<0.01$, and *** represents $p<0.001$.


A
Hmox1
G6pd
Pgd
Relative Gene Expression
ns
***
**
*
6-AN (5μM)
Heme (10μM)
B
Glut1
G6pd
Pgd
Fold mRNA Expression
DHEA
heme

Supplementary Figure 3. Related to Figure 4. A. mRNA expression of *Hmox1*, *G6pd*, and *Pgd* in BMDMs treated with heme and/or 6-aminonicotinamide (2μM) for 6 hours (n = 4). **B.** mRNA expression *Glut1, G6pd*, and *Pgd* in BMDMs treated with heme and/or DHEA (100μM) for 6 hours (n = 4). Data are represented as mean ± SEM. Significance between more than 2 groups was determined by One-Way ANOVA, with post-hoc Tukey's or Dunnett's Multiple Comparison Tests, as appropriate, to determine differences between specific groups. * represents $p<0.05$, ** represents $p<0.01$, and *** represents $p<0.001$.


A
Glut1
G6pd
Pgd
Fold mRNA Expression
SnPPIX
heme
B
Glut1
G6pd
Pgd
Fold mRNA Expression
CORM3
iCORM3
WT
Hif1α-KO
ns

Supplementary Figure 4. Related to Figure 5. A. mRNA expression of *Glut1, G6pd*, and *Pgd* in BMDMs treated with heme (50 μM) and/or the heme oxygenase inhibitor tin protoporphyrin (SnPP, 100 μM) for 6 hours (n = 4). **B.** mRNA expression of *Glut1*, *G6pd*, and *Pgd* in BMDMs isolated from wild-type (WT) or Hif1α-deficient mice loaded with vehicle, 100μM CORM3, or 100μM iCORM3 for 6 hours (n = 4). Data are represented as mean ± SEM. Significance between more than 2 groups was determined by One-Way ANOVA, with post-hoc Tukey's tests to determine differences between specific groups. * represents $p<0.05$, ** represents $p<0.01$, and *** represents $p<0.001$.


A
vehicle
iCORM3 (50μM)
CORM3 (50μM)
Fold mRNA Expression
2.0
1.5
1.0
0.5
0.0
Sdha
mt-Co1
mt-Co3
mt-Nd1
mt-Nd2
mt-Nd5
mt-Nd6
mt-Cytb
B
vehicle
iCORM3 (50μM)
CORM3 (50μM)
Fold mRNA Expression
2.0
1.5
1.0
0.5
0.0
Pfkp
Pgk1
Aldoa
Eno1

Supplementary Figure 5. Related to Figure 6. A. mRNA expression of genes encoding subunits of the mitochondrial electron transport chain in BMDMs treated with vehicle, 50μM CORM3, or 50μM iCORM3 for 6 hours (n = 4). **B.** mRNA expression of glycolysis genes in BMDMs treated with vehicle, 50μM CORM3, or 50μM iCORM3 for 6 hours (n = 4). Data are represented as mean ± SEM.

Supplementary Table 1. Primer sequences used for RT-qPCR.

Gene	Forward	Reverse	Source
Aldoa	GAGGTTCTGGTGACCCTAGC	AATGCTTCCGGTGGACTCATC	PrimerBlast
Aldob	AACCGTTGCCCTCTACCAAG	AGTTAGCCATAGCCCGCTTC	
Aldoc	TCTCTCTTGGGATCAGGGGG	CAGGTGAACCCTTCCTCCAC	
B2m	ATTCACCCCCACTGAGACTG	TGCTATTTCTTTCTGCGTGC	
Blvra	CTGGCTGCACAGAAAGGGAG	GGCGAGAAATGCCACTGAAC	
Blvrb	AGGCACATGGAGTGGACAAG	GGTTGGTCTCCTATGTGTGGG	
Eno1	AGAGTGGGAGGCGCTTAGT	CGGAAGAGACCTTTTGCGGT	
Eno2	TGCAGCTCTTGACTCATCGG	CACCACGGAGATACCTGAGC	
Eno3	CAGCTGCTACCTAGAGGAGAC	TGCTCGGAATCGACCCTTG	
G6pd	TGCACTTTGTCCGGAGTGAT	AGGACAAAATGGCGGTCCAA	Our group[2]
Gapdh	TGAAGGGTGGAGCCAAAAGG	ACTTGGCAGGTTTCTCCAGG	PrimerBlast
Glut1	CGAGGGACAGCCGATGTG	TGCCGACCCTCTTCTTTCAT	
Hk1	ACGATGACTGCGTATCGGTC	CGGGAATACTGTGGGTGCAT	
Hk2	CGCCGGATTGGAACAGAACT	TACTGGTCAACCTTCTGCACTT	
Hk3	CTGAGATGGAGGACACCGC	TTTGGGGCCAGGAGTGTTAC	
Hmox1	ACAGCCCCACCAAGTTCAAA	TCTGCAGGGGCAGTATCTTG	
Pfkp	GTCCCACCCACTTGCAGAAT	AATCTACACGGGGGCCAAAG	
Pgam1	GCAACATCAGCAAGGATCGC	GCCTCTTCGCCTTCACTTCT	
Pgam2	AACATCTGGAAGGGATGTCCG	TTCGTCTCCCAGGAACCTCA	
Pgam5	CCCTGACACCATTAGGTCGG	TACTGCCGTTGTTGAGGGAC	
Pgd	CTCCTCGACTCTGCTTCGTC	CGGCATCTTCTTGTCGTGTC	Our group[2]
Pgk1	CGAGCCTCACTGTCCAAACT	GTCTGCAACTTTAGCGCCTC	PrimerBlast
Pgk2	ATCTGGTTGGATGGGCTTGG	AGAAGCTCAAGACTTGCCCC	
Taldo	CAGATGCCTGCCTACCAAGA	GGAAAGCCTTGCATCAACTTCT	Our group[2]
Tkt	CTACCACGCCATGGAAGGTT	ACTCGGTAGCTGGCTTTGTC	PrimerBlast
Tpi	AGATGAGCTGATTGGCCAGAA	GGGCAGTGCTCATTGTTTGG	
Il6	ACAACGATGATGCACTTGCAG	GCATTGGAAATTCGGGTAGGAA	Our group[3]
Ptgs2	CGGAGAGAGTTCATCCCTGA	ACCTCTCCACCAATGACCTG	Our group[2]
Nos2	CCTGGTACGGGCATTGCT	GCTCATGCCTCCTT	
Il10	ACTACCAAAGCCACAAGGCA	TGGCAACCCAAGTAACCCTTA	PrimerBlast
Il4	CCATATCCACGGATGCGACA	CTGTGGTGTTCTTCGTTGCTG	
Ym1	CTTCCACAGGAGCAGGAATC	GCTCCATGGTCCTTCCAGTA	PrimerBlast
Msr1	CATCACCAACGACCTCAG	ACCAGTTTGTCCAGTAAGC	Our group[4]
Sod2	CTCTAATCAGGACCCATTG	TGCTCTACACTACTATAAACC	PrimerBlast
Gclm	TGGAGCAGCTGTATCAGTGG	AGAGCAGTTCTTTCGGGTCA	Our group[2]
Gss	CGTTCTCAATGTCCTG	AACTTCCTGGTCATCC	PrimerBlast
Gsr	TGTCAAAGGCGTCTATGCTG	GGCTGAAGACCACAGTAGGG	Our group[4]
Txnrd1	GGCTCAGAGGCTGTATGGAG	TCCCAATGGCCAAAAGAAAC	
Srxn1	ACGGTGCACAACGTACCAAT	CTTGGCAGGAATGGTCTCTC	
Il1b	CAAAATACCTGTGGCCTTGG	TACCAGTTGGGGAACTCTGC	
Sdha	GCGGTGGTCACCTTGATCC	CCTCTGTAGAAGCGTCTGAATG	Our group[2]

mt-Co1	TCGCAATTCCTACCGGTGTC	CGTGTAGGGTTGCAAGTCAGC	Ahuja et al., (2016)[5]
mt-Co3	CAAGGCCACCACACTCCTAT	ATTCCTGTTGGAGGTCAGCA	
mt-Nd1	TCCGAGCATCTTATCCACGC	GTATGGTGGTACTCCCGCTG	PrimerBlast
mt-Nd2	AGGGATCCCACTGCACATAG	TGAGGGATGGGTTGTAAGGA	Ahuja et al., (2016)[5]
mt-Nd5	ATAACCGCATCGGAGACATC	GAGGCCAAATTGTGCTGATT	
mt-Nd6	ATGTTGGAAGGAGGGATTGGG	TACCCGCAAACAAAGATCACC	
mt-Cytb	ATTCCTTCATGTCGGACGAG	ACTGAGAAGCCCCCTCAAAT	

Supplementary Table 2, related to Figure 2. Fold changes of metabolites that were significant ($p < 0.05$) or trending toward significant ($p < 0.10$). Red represents a fold increase in heme-loaded BMDMs, green represents a fold decrease in heme-loaded cells.

Metabolite	Fold Change in Heme Loaded BMDMs	Mean Vehicle	Mean Heme	p-value
threonine	**1.30**	0.9608	1.2450	0.0131
alanine	**1.34**	0.9330	1.2526	0.0046
N-acetylalanine	**1.44**	0.8746	1.2592	0.0285
1-methylhistidine	**3.45**	0.3186	1.0979	0.0007
4-imidazoleacetate	**1.19**	1.3054	1.5584	0.0465
lysine	**1.39**	0.9757	1.3586	0.0111
N6-acetyllysine	**4.28**	0.5358	2.2933	0.0013
N6,N6,N6-trimethyllysine	**1.53**	0.8795	1.3493	0.0367
5-hydroxylysine	**1.33**	0.6827	0.9060	0.0176
phenylalanine	**1.27**	1.0140	1.2870	0.0134
tryptophan	**1.37**	1.0383	1.4221	0.0079
leucine	**1.28**	1.0393	1.3319	0.0489
N-acetylleucine	**1.19**	1.2303	1.4615	0.0444
4-methyl-2-oxopentanoate	**2.01**	0.8368	1.6843	0.0276
3-methyl-2-oxobutyrate	**2.95**	0.5739	1.6942	0.0048
valine	**1.32**	0.9829	1.2958	0.0104
methionine	**1.33**	0.9239	1.2318	0.0033
N-acetylmethionine	**1.41**	0.7697	1.0820	0.0243
N-formylmethionine	**1.66**	0.9017	1.4999	0.0001
methionine sulfoxide	**1.43**	0.9719	1.3861	0.0094
cystathionine	**1.30**	0.3968	0.5157	0.0210
N-acetylcysteine	**1.34**	0.9730	1.3062	0.0141
cysteine sulfinic acid	**1.49**	0.7775	1.1601	0.0223
dimethylarginine (SDMA + ADMA)	**1.23**	0.8237	1.0093	0.0413
N-monomethylarginine	**1.16**	0.8900	1.0289	0.0319
5-methylthioadenosine (MTA)	**1.43**	0.8746	1.2491	0.0150
gamma-glutamylvaline	**1.53**	0.8066	1.2310	0.0073
alanylleucine	**3.23**	0.8331	2.6891	0.0007
glycylisoleucine	**3.22**	0.7486	2.4139	0.0001
glycylleucine	**4.15**	0.7415	3.0740	0.0001
glycylvaline	**2.51**	0.8207	2.0570	0.0002
isoleucylglycine	**1.88**	0.8946	1.6824	0.0016
leucylalanine	**3.82**	0.7865	3.0071	0.0019
leucylglycine	**5.70**	0.7395	4.2135	0.0003
phenylalanylalanine	**2.30**	0.7799	1.7925	0.0065
phenylalanylglycine	**1.55**	1.0400	1.6130	0.0088
prolylglycine	**1.29**	0.7635	0.9873	0.0428
threonylphenylalanine	**4.45**	0.8668	3.8540	0.0008
tyrosylglycine	**1.68**	0.8161	1.3691	0.0075
valylglutamine	**5.01**	0.7448	3.7278	0.0005
valylglycine	**4.23**	0.7473	3.1594	0.0001
valylleucine	**3.71**	0.8759	3.2469	0.0010
leucylglutamine*	**4.20**	0.7556	3.1733	0.0003
dihydroxyacetone phosphate (DHAP)	**1.69**	0.7343	1.2376	0.0375
3-phosphoglycerate	**2.86**	1.1033	3.1537	0.0225

phosphoenolpyruvate (PEP)	**2.80**	1.4224	3.9793	0.0159
lactate	**1.62**	1.2849	2.0828	0.0004
glycerate	**2.29**	0.8748	2.0006	0.0074
6-phosphogluconate	**4.14**	0.6647	2.7490	0.0315
ribulose/xylulose 5-phosphate	**1.86**	0.9390	1.7478	0.0005
ribose	**1.41**	0.7783	1.0946	0.0419
UDP-glucose	**2.42**	0.7175	1.7347	0.0002
UDP-glucuronate	**1.32**	0.9550	1.2627	0.0312
2-hydroxystearate	**2.36**	1.4281	3.3699	0.0376
N-stearoyltaurine	**1.44**	1.3176	1.8914	0.0316
inositol 1-phosphate (I1P)	**3.60**	0.5655	2.0363	0.0002
glycerophosphorylcholine (GPC)	**1.63**	0.7582	1.2370	0.0001
glycerophosphoethanolamine	**1.12**	0.9737	1.0922	0.0362
1-palmitoyl-2-oleoyl-GPC (16:0/18:1)	**1.23**	1.0443	1.2833	0.0400
1-stearoyl-2-oleoyl-GPC (18:0/18:1)	**1.30**	0.9638	1.2486	0.0032
1-palmitoyl-2-oleoyl-GPG (16:0/18:1)	**1.23**	0.9711	1.1915	0.0059
1-palmitoyl-2-oleoyl-GPE (16:0/18:1)	**1.28**	0.9628	1.2281	0.0031
1-stearoyl-2-oleoyl-GPE (18:0/18:1)	**1.22**	0.9259	1.1308	0.0110
1-palmitoyl-2-oleoyl-GPS (16:0/18:1)	**1.21**	0.9869	1.1935	0.0256
1-oleoyl-arachidonoyl-GPI (18:1/20:4)*	**1.27**	0.9327	1.1849	0.0166
1-palmitoyl-2-gamma-linolenoyl-GPC (16:0/18:3n6)*	**1.24**	1.0208	1.2688	0.0154
1-stearoyl-GPG (18:0)	**3.12**	0.8325	2.6007	0.0447
glycerophosphoglycerol	**1.44**	1.0429	1.5048	0.0046
diacylglycerol (12:0/18:1, 14:0/16:1, 16:0/14:1) [2]*	**1.57**	1.0552	1.6616	0.0259
oleoyl-linoleoyl-glycerol (18:1/18:2) [2]	**1.61**	0.7820	1.2564	0.0034
oleoyl-arachidonoyl-glycerol (18:1/20:4) [2]*	**1.68**	0.8515	1.4346	0.0122
linoleoyl-linoleoyl-glycerol (18:2/18:2) [1]*	**1.91**	0.8654	1.6525	0.0339
palmitoyl-dihomo-linolenoyl-glycerol (16:0/20:3n3 or 6) [2]*	**1.81**	0.6448	1.1666	0.0048
palmitoyl-oleoyl-glycerol (16:0/18:1) [1]*	**1.52**	0.8418	1.2790	0.0198
palmitoyl-oleoyl-glycerol (16:0/18:1) [2]*	**1.56**	0.8829	1.3791	0.0190
palmitoyl-linoleoyl-glycerol (16:0/18:2) [2]*	**1.54**	0.9036	1.3921	0.0131
stearoyl-linoleoyl-glycerol (18:0/18:2) [2]*	**1.78**	0.7522	1.3400	0.0062
oleoyl-oleoyl-glycerol (18:1/18:1) [1]*	**3.59**	0.3447	1.2366	0.0034
oleoyl-oleoyl-glycerol (18:1/18:1) [2]*	**1.80**	0.6532	1.1781	0.0358
phytosphingosine	**1.48**	0.7835	1.1566	0.0169
sphingomyelin (d18:1/18:1, d18:2/18:0)	**1.33**	1.0022	1.3372	0.0257
sphingosine	**1.94**	0.5664	1.1015	0.0003
sphingomyelin (d18:1/14:0, d16:1/16:0)*	**1.23**	0.9324	1.1502	0.0309
sphingomyelin (d18:2/16:0, d18:1/16:1)*	**1.28**	0.8686	1.1103	0.0262
sphingomyelin (d18:1/20:1, d18:2/20:0)*	**1.30**	1.0238	1.3354	0.0148
sphingomyelin (d18:1/20:0, d16:1/22:0)*	**1.23**	0.9151	1.1300	0.0495
sphingomyelin (d18:1/21:0, d17:1/22:0, d16:1/23:0)*	**1.38**	0.7951	1.0978	0.0428
cholesterol	**1.20**	0.9703	1.1629	0.0481
deoxycholate	**1.66**	1.1014	1.8295	0.0079
adenosine 3'-monophosphate (3'-AMP)	**2.88**	0.6032	1.7347	0.0104
adenosine	**1.26**	0.8619	1.0855	0.0488
guanosine 3'-monophosphate (3'-GMP)	**7.03**	0.3144	2.2106	0.0011
uridine 3'-monophosphate (3'-UMP)	**4.81**	0.4226	2.0343	0.0071
N-acetyl-beta-alanine	**1.81**	1.2955	2.3409	0.0023
adenosine 5'-diphosphoribose (ADP-ribose)	**5.35**	3.7680	20.1708	0.0138
heme	**1.87**	0.4916	0.9195	0.0347
bilirubin (Z,Z)	**164.72**	0.3772	62.1333	0.0000
bilirubin (E,E)*	**2.38**	0.3640	0.8649	0.0364
biliverdin	**1.46**	0.6443	0.9396	0.0168

serine	**1.17**	0.9821	1.1495	0.0589
histidine	**1.29**	0.9665	1.2514	0.0671
tyrosine	**1.26**	1.0750	1.3529	0.0660
O-methyltyrosine	**1.32**	0.9258	1.2206	0.0831
N-acetylmethionine sulfoxide	**1.51**	0.8299	1.2564	0.0883
4-acetamidobutanoate	**1.48**	0.2413	0.3574	0.0903
ophthalmate	**1.30**	0.2269	0.2943	0.0577
gamma-glutamyl-epsilon-lysine	**1.17**	0.8453	0.9907	0.0574
pyruvate	**1.61**	0.8245	1.3249	0.0593
lactose	**1.57**	1.3297	2.0932	0.0645
N-acetylglucosamine 6-phosphate	**1.49**	0.7225	1.0776	0.0686
alpha-ketoglutarate	**1.22**	0.8319	1.0118	0.0581
acetylphosphate	**1.45**	1.0170	1.4780	0.0785
acetylcarnitine	**1.31**	0.4619	0.6034	0.0523
oleoylcarnitine	**1.42**	0.3042	0.4325	0.0836
2-hydroxypalmitate	**1.73**	1.6225	2.8030	0.0899
1-palmitoyl-2-linoleoyl-GPC (16:0/18:2)	**1.18**	1.1171	1.3221	0.0543
1-palmitoyl-2-palmitoleoyl-GPC (16:0/16:1)*	**1.38**	1.1010	1.5172	0.0635
1-oleoyl-2-linoleoyl-GPC (18:1/18:2)*	**1.21**	1.0386	1.2587	0.0723
1-palmitoyl-2-linoleoyl-GPE (16:0/18:2)	**1.21**	1.1865	1.4400	0.0626
1-stearoyl-2-linoleoyl-GPE (18:0/18:2)*	**1.20**	1.1423	1.3705	0.0882
1-palmitoleoyl-2-linoleoyl-GPC (16:1/18:2)*	**1.22**	0.9815	1.1960	0.0952
1-arachidonoyl-GPE (20:4n6)*	**1.89**	1.2026	2.2711	0.0776
1-oleoyl-GPG (18:1)*	**1.72**	1.0380	1.7894	0.0588
1-linoleoyl-GPG (18:2)*	**1.56**	1.0838	1.6893	0.0575
1-arachidonylglycerol (20:4)	**1.52**	0.8088	1.2324	0.0915
2-arachidonoylglycerol (20:4)	**1.68**	0.8076	1.3576	0.0533
diacylglycerol (14:0/18:1, 16:0/16:1) [1]*	**2.15**	0.6076	1.3040	0.0814
diacylglycerol (14:0/18:1, 16:0/16:1) [2]*	**1.69**	0.7954	1.3420	0.0603
diacylglycerol (16:1/18:2 [2], 16:0/18:3 [1])*	**1.21**	1.2054	1.4637	0.0697
linoleoyl-arachidonoyl-glycerol (18:2/20:4) [2]*	**1.90**	0.8001	1.5195	0.0881
stearoyl sphingomyelin (d18:1/18:0)	**1.21**	1.0679	1.2960	0.0961
N-palmitoyl-sphingosine (d18:1/16:0)	**1.29**	0.8746	1.1296	0.0987
sphingomyelin (d18:1/24:1, d18:2/24:0)*	**1.17**	0.9463	1.1062	0.0643
glycosyl-N-palmitoyl-sphingosine	**1.22**	0.9986	1.2162	0.0818
7-hydroxycholesterol (alpha or beta)	**1.73**	1.0353	1.7903	0.0547
guanosine	**1.41**	0.8548	1.2024	0.0752
orotate	**1.39**	1.5010	2.0866	0.0695
uridine 5'-monophosphate (UMP)	**1.38**	0.9309	1.2815	0.0583
thiamin (Vitamin B1)	**1.20**	1.0502	1.2633	0.0958
gluconate	**1.81**	0.8468	1.5295	0.0585
erythritol	**1.20**	0.2690	0.3237	0.0915
methyl glucopyranoside (alpha + beta)	**1.95**	0.8488	1.6516	0.0624
penicillin G	**1.29**	0.9905	1.2798	0.0856
betaine	**0.58**	0.5165	0.3005	0.0000
aspartate	**0.55**	0.8776	0.4865	0.0044
gamma-carboxyglutamate	**0.64**	0.6748	0.4324	0.0084
glutarate (pentanedioate)	**0.61**	0.4826	0.2921	0.0135
C-glycosyltryptophan	**0.80**	0.9930	0.7895	0.0365
hypotaurine	**0.53**	0.8739	0.4639	0.0097
taurine	**0.67**	1.0238	0.6875	0.0038
guanidinoacetate	**0.68**	1.4098	0.9541	0.0023
galactose 1-phosphate	**0.66**	1.5060	0.9865	0.0296
N-acetylglucosaminylasparagine	**0.58**	1.0037	0.5802	0.0350

adipate	**0.63**	0.3005	0.1886	0.0096
choline phosphate	**0.52**	1.0060	0.5269	0.0003
phosphoethanolamine	**0.75**	1.1207	0.8423	0.0173
arachidonoylcholine	**0.69**	0.6911	0.4746	0.0435
oleoylcholine	**0.44**	0.9236	0.4050	0.0008
palmitoloelycholine	**0.38**	1.0070	0.3780	0.0004
1-palmitoyl-GPC (16:0)	**0.60**	1.0598	0.6367	0.0293
1-stearoyl-GPE (18:0)	**0.75**	1.0018	0.7499	0.0474
N-palmitoyl-sphinganine (d18:0/16:0)	**0.60**	1.1834	0.7065	0.0464
palmitoylcholine	**0.45**	1.3891	0.6274	0.0292
inosine 5'-monophosphate (IMP)	**0.47**	2.8592	1.3521	0.0057
adenosine 2'-monophosphate (2'-AMP)	**0.40**	0.6011	0.2390	0.0313
adenosine 3',5'-diphosphate	**0.45**	1.5368	0.6850	0.0010
beta-alanine	**0.62**	0.8995	0.5591	0.0023
nicotinamide riboside	**0.75**	1.2831	0.9571	0.0187
5-methyltetrahydrofolate (5MeTHF)	**0.34**	0.7682	0.2586	0.0322
retinol (Vitamin A)	**0.57**	0.5515	0.3143	0.0032
pyridoxine (Vitamin B6)	**0.77**	1.3394	1.0304	0.0492
imidazole propionate	**0.76**	0.8418	0.6421	0.0597
phenol sulfate	**0.73**	0.4947	0.3599	0.0712
creatinine	**0.85**	0.4659	0.3943	0.0917
mesaconate (methylfumarate)	**0.68**	0.6576	0.4492	0.0839
1-stearoyl-GPC (18:0)	**0.69**	0.9166	0.6301	0.0632
1-palmitoyl-GPE (16:0)	**0.68**	1.0048	0.6875	0.0604
1-stearoyl-GPI (18:0)	**0.63**	1.0337	0.6563	0.0599
urate	**0.72**	1.3816	0.9961	0.0524
adenylosuccinate	**0.53**	0.6399	0.3397	0.0787
beta-guanidinopropanoate	**0.62**	1.2584	0.7850	0.0563

Supplementary References

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